

Image analysis

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 An abbreviated version of this protocol was published in Science in Nov 2020

Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity

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Detailed protocol

Image visualization was performed using Fiji software and Cell Profiler. The numbers of NRP1+ positive neurons in the mouse main olfactory epithelium (MOE) and brain were manually counted from three representative sections of three independent animals. To measure the infectivity of cultured cells, three randomly selected areas per coverslip were imaged. Automated counting was performed with a plugin for Fiji (uploaded to this website). The percentage of GFP positive cells was determined by dividing the number of GFP+ nuclei by the number of Hoechst+ nuclei. The same threshold value was applied to all the coverslips of an independent experiment. To measure the AgNP uptake in cultured HEK293-T cells, three randomly selected areas per coverslip were imaged. AgNP positive cells were determined the following way: each cell was identified by the positivity for the nuclear staining Hoechst 33342 and the actin-binding toxin phalloidin. Hoechst+ nuclei were used to define the cell ROI. Within each ROI, the area occupied by the signal of AgNPs was measured and expressed as area fraction. ROIs with an area fraction higher than 30% were considered AgNP+ cells. The percentage of AgNP+ cells was determined by dividing the number of AgNP+ cells by the number of Phalloidin+ cells. All samples were analyzed at the same threshold values. For quantification of AgNPs internalization in Caco-2 15 cells, after automated imaging of 96-well plates, the fraction of AgNP-positive cells was determined using Cell Profiler-3 software. Nuclei and cell cytoplasm were identified using the fluorescent signal of nuclei stained with DNA staining Hoechst and whole cells stained with actin marker phalloidin-A647. A threshold of fluorescence was determined such that no positive cells were identified in samples that did not receive the AgNPs. The accuracy of detection was visually inspected in representative images from each experiment before analyzing the whole data set. Nuclei were detected using the Otsu algorithm of CellProfiler ([www.Cellprofiler.org](http://www.cellprofiler.org)).

For the in vivo analysis three representative sections from four independent animals were stained with the specific antibodies. For each tissue a representative area was selected and the area occupied by AgNP particles with a size higher than 1.8

Related files

 GFPcells plugin.docx



How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Cantuti-Castelvetri, L. (2021). Image analysis. Bio-protocol Preprint. bio-protocol.org/prep794.
2. Cantuti-Castelvetri, L., Ojha, R., Pedro, L. D., Djannatian, M., Franz, J., Kuivanen, S., Meer, F. V. D., Kallio, K., Kaya, T., Anastasina, M., Smura, T., Levanov, L., Szivovicza, L., Tobi, A., Kallio-Kokko, H., Österlund, P., Joensuu, M., Meunier, F. A., Butcher, S. J., Winkler, M. S., Mollenhauer, B., Helenius, A., Gokce, O., Teesalu, T., Hepojoki, J., Vapalahti, O., Stadelmann, C., Balistreri, G. and Simons, M. (2020). Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science 370(6518). DOI: [10.1126/science.abd2985](https://doi.org/10.1126/science.abd2985)

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